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14. ABSTRACT
The purpose of this study is to develop and characterize a surface plasmon resonance (SPR)-based assay that can specifically detect binding of APF to its cellular receptor, cytoskeleton associated protein 4 (CKAP4), immobilized on a sensor chip surface and to test the ability of this SPR-based assay to discriminate and measure the concentration of APF in urine from well-defined IC patients vs. age-matched, asymptomatic controls. In the first year of study, we completed all of the pre-clinical study components for Aim 2 and received all of the necessary regulatory approvals. The infrastructure for the study was established, including hiring relevant personnel as well as establishing SOPs and procuring supplies to ensure consistency across sites. Patient recruitment has begun and is on track. Considerable progress has been made toward the development, characterization, and eventual testing of clinical samples by the SPR assay. The focus in year one has been on development of the SPR assay using CKAP4 as a biosensor to detect APF. Our results demonstrate that we have successfully optimized rCKAP4 activity and immobilization with sufficient binding efficiency to detect and quantitate APF in a purified system. A parallel approach using an APF mAb as a biosensor was pursued to enhance sensitivity of the assay; it offers an alternate strategy to specifically measure APF in urine with the goal of developing a non-invasive, point-of-care diagnostic test for IC.

15. SUBJECT TERMS
APF, CKAP4, IC

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INTRODUCTION:

Interstitial cystitis (IC) is a chronic, debilitating bladder disease that affects mostly women and is frequently misdiagnosed due to lack of a non-invasive test to detect the disease[1-6]. While the cause remains unknown, biomarkers for IC have been described, including antiproliferative factor (APF), a glycopeptide that is detectable in the urine of 95-97% of IC patients vs. normal controls[7-9]. Validation of APF as a biomarker and etiologic agent for IC has been hindered by the absence of robust assays to detect and measure its concentration in patient urine. The purpose of this study is 1) to develop and characterize a surface plasmon resonance (SPR)-based assay that can specifically detect binding of APF to its cellular receptor, cytoskeleton associated protein 4 (CKAP4), immobilized on a sensor chip surface and 2) to test the ability of this SPR-based assay to discriminate and measure the concentration of APF in urine from well-defined IC patients vs. age-matched, asymptomatic controls. Approximately 90 patients will participate in this study. Urine specimens from 30 IC patients will be collected by Dr. Phillip Hanno and his staff during routine office visits to the Penn Urology IC Clinic at the University of Pennsylvania Hospital for the management of IC. Urine specimens from asymptomatic controls will be collected at TCMC by Betsy Mead, the clinical research coordinator. All processed urine samples will be shipped on dry ice to Dr. Susan Keay, who will blindly test the fresh urine specimens for APF activity by ^{3}H-thymidine incorporation in addition to 30 banked frozen specimens (from another 15 IC female patients and 15 age-matched, asymptomatic controls) for comparison to results obtained from the SPR assay. We expect that the SPR-assay will overcome current barriers associated with validation of APF as a diagnostic biomarker for IC by being able to specifically detect the presence of APF in urine and accurately quantitate its levels for the first time. This would meet a critical need for an affirmative, diagnostic test for IC with the advantages of being rapid, specific, and non-invasive; further, it would present major learning opportunities for advancing our knowledge about the contribution of APF to IC.

KEYWORDS:

APF:	antiproliferative factor
CKAP4:	cytoskeleton associated protein 4
ED:	extracellular domain
FL-CKAP4:	full-length cytoskeleton associated protein 4
HT-CKAP4:	histidine (x6) tagged-cytoskeleton associated protein 4
IC:	interstitial cystitis
kDa	kilodalton
KLH:	keyhole limpet hemocyanin
mAb:	monoclonal antibody
NTA:	nitrilotriacetic acid
PAGE:	polyacrylamide gel electrophoresis
PBST:	phosphate buffered saline containing Tween-20
SDS:	sodium dodecyl sulfate
SPR:	surface plasmon resonance

OVERALL PROJECT SUMMARY:

The original Statement of Work indicated two specific aims or tasks to be accomplished during the 3-year funding period, with emphasis on the first aim in the first two years and the second aim in the final year: 1) Develop and characterize a SPR-based assay employing a CKAP4 immobilized biosensor to detect APF 2) Determine the ability of the SPR-based assay to detect APF in urine from patients with IC.

In the first year of the grant reporting period, we formulated the protocol for the clinical study, developed the informed consent documents and other documents required by regulatory bodies. All of these were submitted for regulatory review to the IRBs at the Commonwealth Medical College (Wright Center for Graduate Medical Education), the University of Pennsylvania, the University of Maryland, the US Army

Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), and the Human Research Protection Office (HRPO) and approval was obtained. We also established the infrastructure for the study by recruiting staff for the post-doctoral and research technician positions, obtaining required shipping infectious substances (IATA and DOT) certification for appropriate personnel, procuring shipping supplies for clinical specimens that are compatible with IATA regulations as well as other protocol-related supplies to be used at each study site. A standard operating procedure was also established for collecting and processing fresh urine specimens across each study site. Once these regulatory and infrastructural issues were accomplished, we began recruiting patients for the study at both the University of Pennsylvania and at TCMC. In addition, we have predominantly focused our effort in the first year on development and characterization of the SPR-based assay.

Below we present the original SOWs (in italics) and our accomplishments over the first year.

SOW – Specific Aim 1/Task 1: *Develop and characterize a SPR-based assay employing a CKAP4 immobilized biosensor to detect APF.* During this annual reporting period, effort was expended primarily on Task 1a under Specific Aim 1. We have made substantial progress as detailed below.

1a: *Optimization of rCKAP4 activity and immobilization on sensor chip surface (months 1 -12).* In order to develop a robust method to immobilize CKAP4 onto a sensor chip surface to measure APF binding, optimization of recombinant CKAP4 (rCKAP4) activity was pursued. Various strategies were employed to promote CKAP4's most physiologically relevant conformation in order to obtain improved APF binding efficiency. In our initial approach, we generated two recombinant, full-length (FL)-CKAP4 constructs—one tagged with His6 at its N-terminus and another tagged with His6 at its C-terminus—in order to determine whether FL-CKAP4 could improve APF binding response over the truncated rCKAP4 used in our preliminary studies. Both constructs were expressed successfully in HEK293T cells and purified with sufficient purity and concentration for sensor chip immobilization (Figure 1).

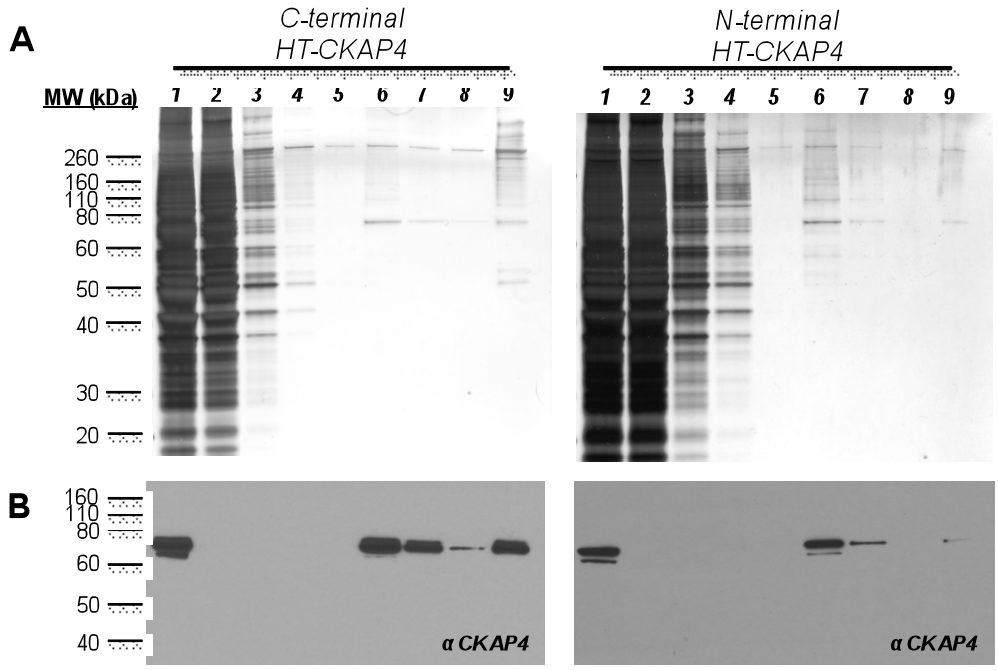


Figure 1. Purification of FL-CKAP4 using GE HiTrap TALON crude resin. Lane assignments are as follows: Lane 1 is pre-purification lysate; lane 2 is post-purification lysate (flow-through); lanes 3-5 are washes; lanes 6-8 are elutions 1-3 respectively with 500 mM imidazole present in elution buffer; and lane 9 is post-purification resin. (A) Protein staining with Pierce Silver Stain Kit (B) Western blot analysis using anti-CKAP4 antibodies. Column 1: Purification of C-terminal His-tagged FL-CKAP4. Column 2: Purification of N-terminal His-tagged FL-CKAP4.

Attempts to immobilize either FL-CKAP4 protein on the NTA sensor chip were successful, as both proteins were strongly recognized by a CKAP4 antibody, indicating high activity. However, when tested by SPR, FL-CKAP4 did not improve APF binding response over the truncated rCKAP4 (Figure 2). We then concentrated our efforts on optimization of the truncated rCKAP4 to generate stronger binding. Using the extracellular domain (ED) of CKAP4 expressed in bacterial system with N-terminal His tag,

we found that the soluble fraction of HT-CKAP4-ED showed clear specific binding, whereas the inclusion body fraction did not show binding activity to APF (Figure 3A and 3B). Collectively, these results suggested that for optimal binding activity to APF, CKAP4 needs to be in a native conformation, and that the truncated form produces stronger binding.

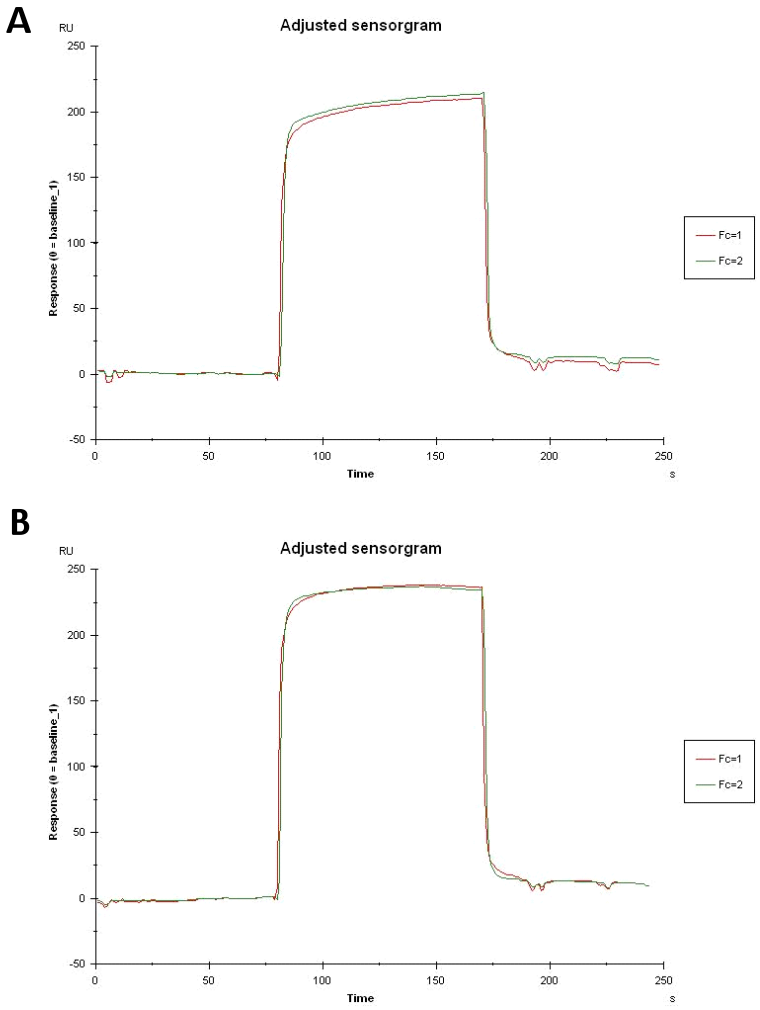


Figure 2. Binding of FL-CKAP4 protein to APF. APF was immobilized to a CM5 chip by amine coupling. Fc1 channel is with control APF; Fc2 channel is with APF. A) FL-CKAP4-HT (His6 tagged at C-terminus) purified from HEK293T cell was used as the analyte. B) HT-FL-CKAP4 (His6 tagged at N-terminus).

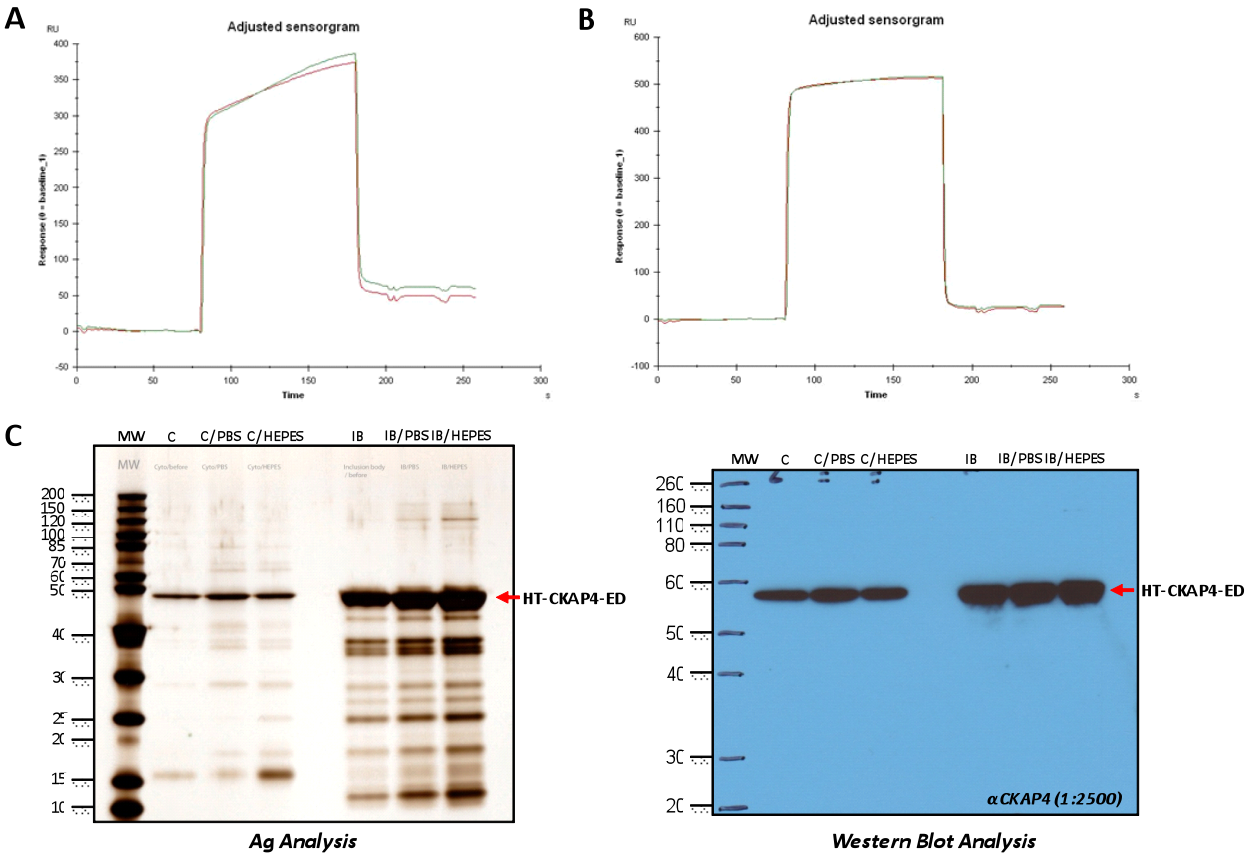


Figure 3. The extracellular domain of CKAP4 binds to APF specifically . HT-CKAP4-ED was expressed and purified from bacterial system and used as the analyte in SPR assay. APF was immobilized on CM5 (Fc2) through amine-coupling as the ligand. The Fc1 channel was immobilized with APF control peptide as a reference. The interaction was measured by Biacore X-100 Plus and the sensorgram was shown. **(A).** APF binding to purified soluble fraction of HT-CKAP4-ED; **(B).** APF binding to purified inclusion body fraction of HT-CKAP4-ED. **(C).** The purity of purified HT-CKAP4 was analyzed SDS-PAGE followed by silver staining and Western blot analysis.

Based on these results, we focused solely on the extracellular domain of CKAP4 in an effort to identify the primary APF binding domain and thus establish a robust binding assay with much higher sensitivity. Using structural prediction software, we analyzed the CKAP4 ED (Figure 4A) and determined the optimal deletion strategy for generating mutants of this region while preserving structural and functional domains (Figure 4B). Four CKAP4 deletion mutant constructs consisting of amino acids 106-602, 127-360, 361-524, and 525-602 were generated by PCR and subcloned into the pET15b bacterial expression vector. All of the mutants (tagged at N-terminus with His_6) were expressed successfully in bacteria.

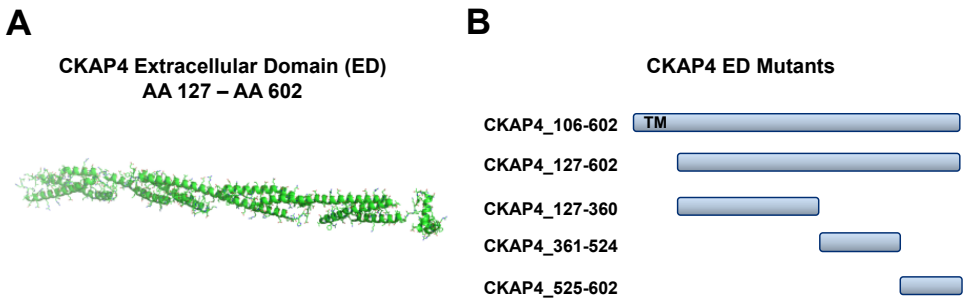
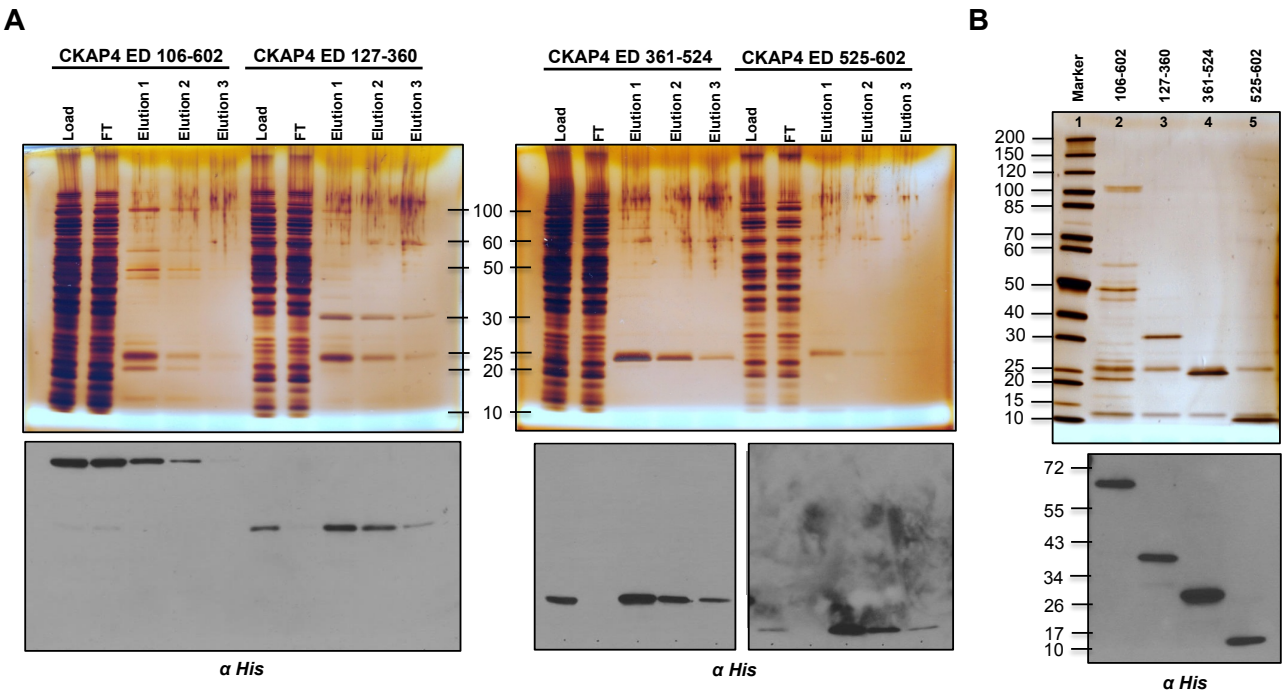


Figure 4. CKAP4 extracellular domain (ED) deletion mutant strategy. (A). Predicted structure for CKAP4 extracellular domain, not including the transmembrane domain (amino acids 127-602) **(B)**. CKAP4 ED mutants were generated to identify the primary APF binding domain, while preserving structural and functional domain features.

The strategy for purifying and dialyzing these mutant proteins has been successfully established (Figure 5A and 5B). By changing the immobilization strategy, a CKAP4 ED mutant (361- 524) was immobilized to a CM5 chip via amine-coupling. As shown in Figure 5C, it exhibited specific binding to active APF, indicating the high potential in identifying and quantitating APF in urine samples. The testing of three additional mutants is currently underway with the goal of identifying the primary APF binding domain and establishing a robust binding assay with even higher sensitivity.



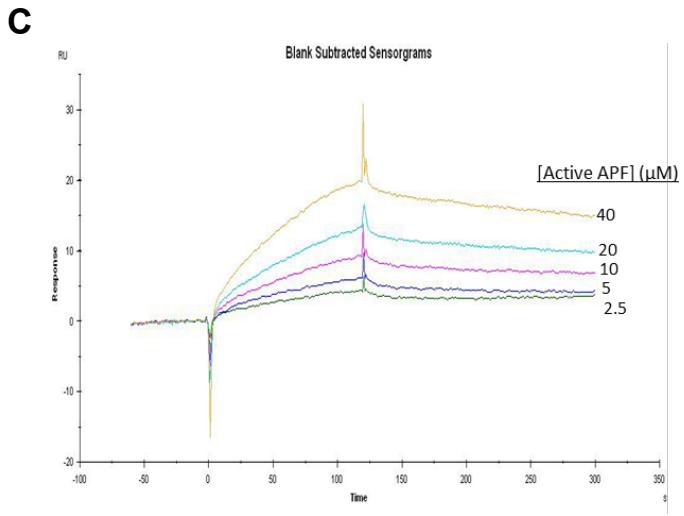
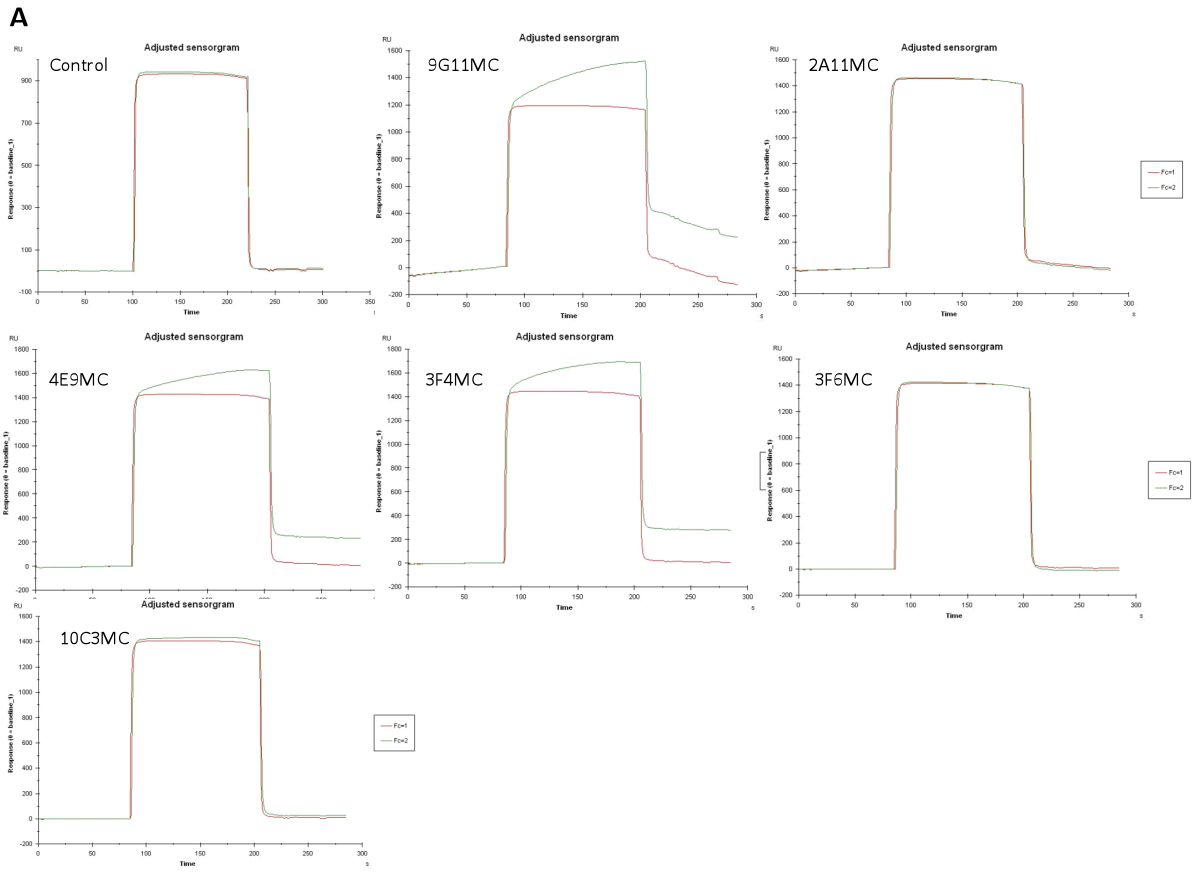


Figure 5. Purification of CKAP4 extracellular domain (ED) mutants using TALON Superflow resin. **(A)** Purification of CKAP4 ED mutants. All mutants are tagged with His_6 at the N-terminus and purification was confirmed by protein staining with the Pierce Silver Stain Kit (upper panels) and Western Blot analysis (bottom panels) using an anti-His antibody. **(B)** CKAP4 ED mutants post-dialysis. Elution 1-3 for each mutant were pooled and dialyzed into PBS buffer. Lane assignments are as follows: Lane 1 is the protein standard; Lane 2 is post-dialysis, purified CKAP4 mutant 106-602; Lane 3 is post-dialysis purified CKAP4 mutant 127-360; Lane 4 is post-dialysis purified CKAP4 mutant 361-524; Lane 5 is post-dialysis purified CKAP4 mutant 525-602. **(C)** Binding of CKAP4 deletion mutant 361-524 to active APF by the SPR assay. Purified CKAP4 deletion mutant 361-524 was immobilized to the Fc2 channel of CM5 chip by amine-coupling as the ligand; the Fc1 channel was blank-immobilized as the control. A series of concentrations of active APF were injected as the analyte to bind the CKAP4 mutant for the kinetic assay, and the adjusted sensorgrams (Fc2-Fc1) are shown.

Based our findings using CKAP4 variants, we sought to test an alternative approach that may significantly increase the sensitivity of our SPR assay. This new methodology utilizes monoclonal anti-APF antibody as the biosensor in the SPR assay to detect APF as the analyte. Using keyhole limpet hemocyanin (KLH)-conjugated APF on the surface of a CM5 chip, we screened 13 primary hybridoma clones (previously generated by our laboratory) for APF sensitivity using the SPR assay (Figure 6A). We identified 6 clones that demonstrate high binding affinity to APF. This result was confirmed by antibody purification (Figure 6B and 6C). Two of these APF hybridomas (7E11 and 6E7) were selected for further subcloning based on their performance in ELISA and SPR analyses. These crude hybridomas were subcloned by limiting dilution to isolate single clones with unique antigenicity.

A



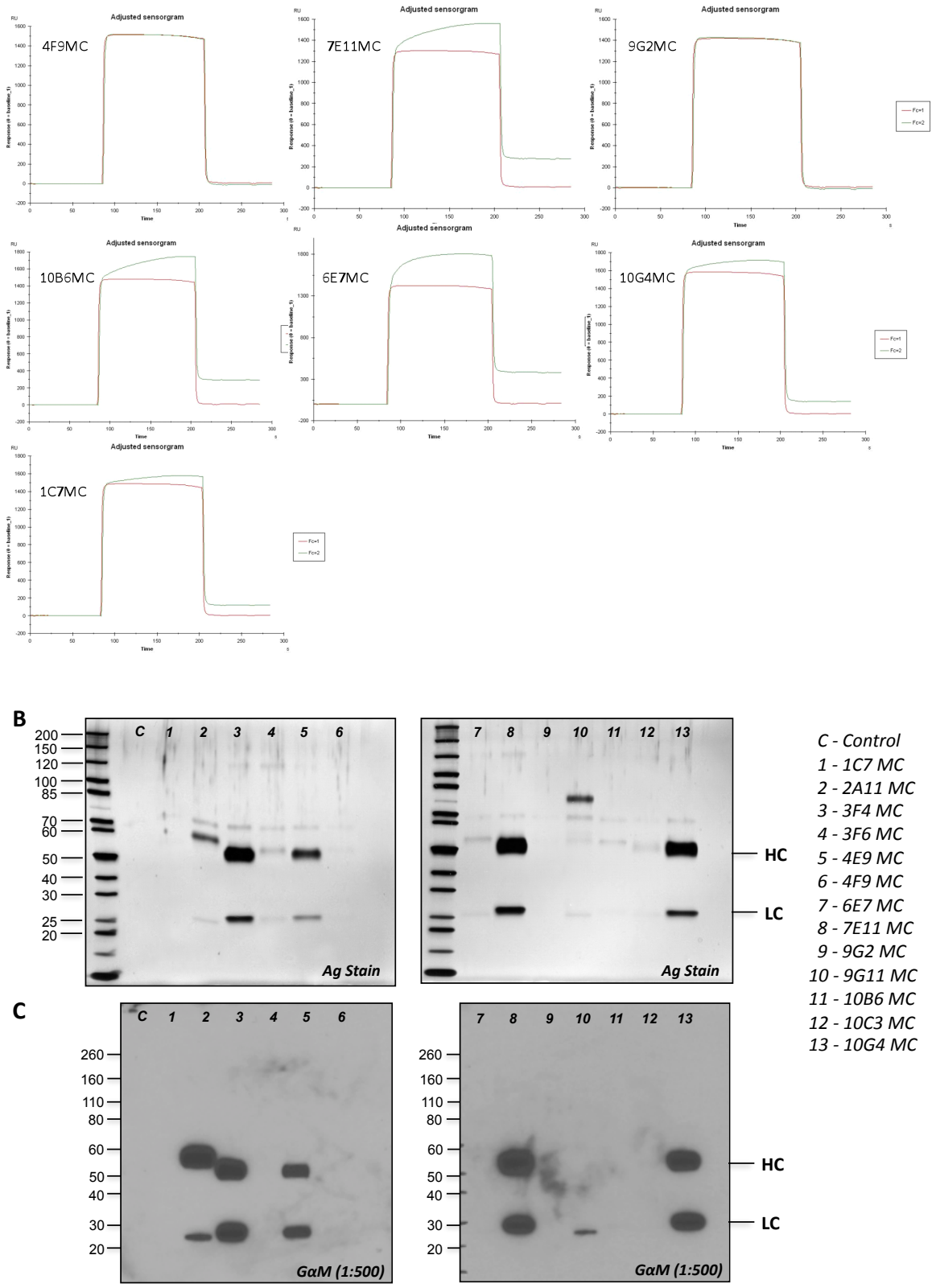


Figure 6. Screening of APF monoclonal antibody hybridomas by SPR assay and antibody purification. (A) Cell culture lysates from 13 primary hybridomas were analyzed by SPR to measure their binding activities to APF. KLH-APF was immobilized on a CM5 chip (Fc2) as the ligand by amine-coupling reaction; Fc1 was the control. The lysate was diluted 2-fold by PBS/0.01% Tween-20 for the binding assay; sensorgrams are shown. (B) The monoclonal antibody hybridomas were purified by protein A/G bead (Pierce) from 1 ml of lysate and identified by SDS-PAGE followed by silver staining. (C) The same gel shown in B was transferred to PVDF membrane for Western blot analysis using anti-mouse IgG secondary antibody.

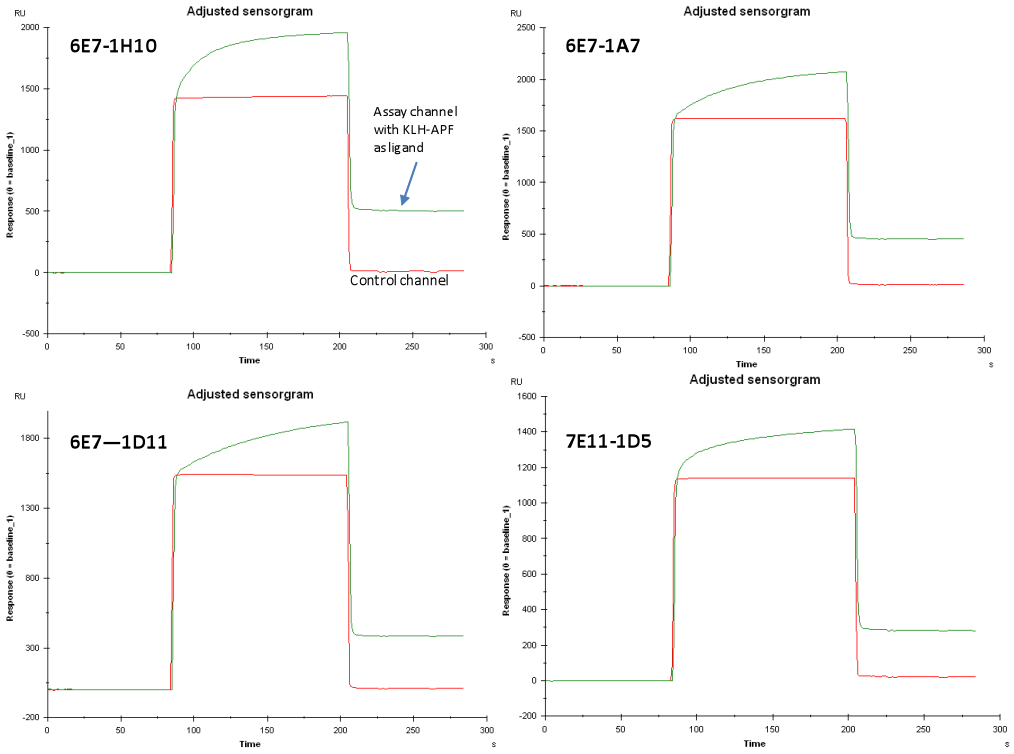
During the final 6-well cloning stage, 12 clones from the 7E11 (IgG1, k chain) and 6E7 (IgG3, k chain) hybridomas were screened by ELISA, Octet, and SPR to determine their specificity for APF, their antibody quantitation, and their secretion (Figure 7A and 7B).

A

Clones	Elisa O.D. Whole molecule 2 °	Elisa O.D. IgM mu-chain 2 °	Elisa O.D. Gamma- chain IgG 2 °	Octet QKe IgG Qaunt. IgG conc. ug/ml.
7E11-1D5	2.586	0.073	1.390	77.4
7E11-1C11	2.550	0.071	1.353	54.5
7E11-1C4	2.758	0.074	1.600	53.6

Clones	Elisa O.D. Whole molecule 2 °	Elisa O.D. IgM mu-chain 2 °	Elisa O.D. Gamma- chain IgG 2 °	Octet QKe IgG Qaunt. IgG conc. ug/ml.
6E7-1H10	3.069	0.072	1.334	73.2
6E7-1A7	3.041	0.076	1.486	45.1
6E7-1D11	3.034	0.074	1.361	43.8

B



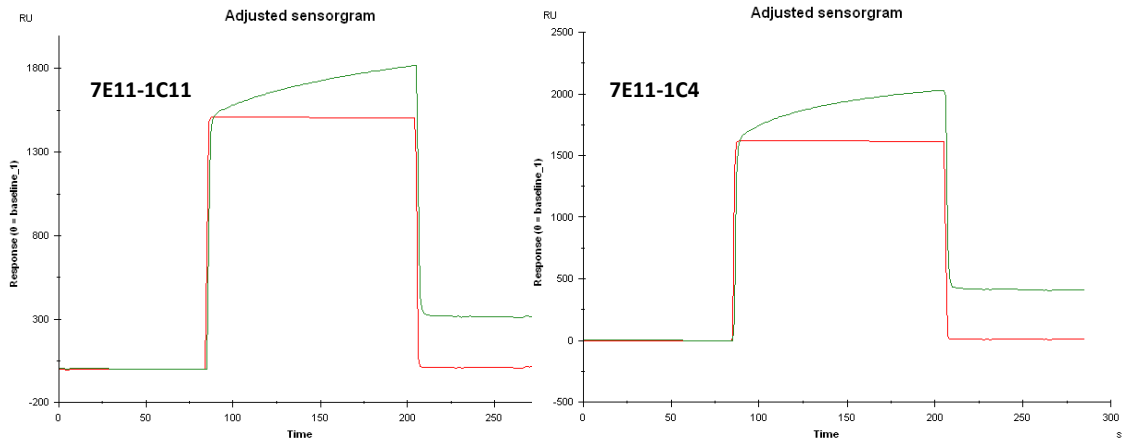


Fig. 7. Screening of subcloned of APF monoclonal antibody hybridomas by ELISA, Octet, and SPR analysis. Crude APF mAb hybridomas were subcloned by limiting dilution to isolate single clones with unique antigenicity. At each stage of culture—96-well, 24-well, and 6-well—the cell culture supernatants were screened by ELISA and SPR to identify clones with high affinity and optimal kinetics for further cloning. During the final 6-well cloning stage, 12 clones each from the 7E11 hybridoma (IgG1, κ chain) and 6E7 hybridoma (IgG3, κ chain) were screened by ELISA and SPR. An Octet assay was also performed on all 24 clones to determine their antibody quantitation and secretion. All 24 clones showed positive reactivity against APF, with varying binding kinetics. Data analysis for three clones from each hybridoma are shown in this figure. **(A)**. Table listing the ELISA results with 2nd antibody against total IgG and γ heavy chain, respectively; Octet results are also shown. **(B)**. SPR assay sensorgrams for the corresponding clones listed in the table. Two types of binding kinetics are shown which could exhibit different applicability in future APF detection.

Based on the success of these results, five clones from the 7E11 hybridoma and three from the 6E7 hybridoma were selected for large-scale production and purification (Figure 8A). All five purified 7E11 monoclonal antibodies displayed specific activity against APF-KLH in a dot blot assay (Figure 8B). Most importantly, the 7E11-1C3 clone showed specific binding to the active form of APF by the SPR assay. Testing of the other mAb clones is currently underway, and the detailed binding conditions are being optimized. This significant result indicates the potential success of generating an APF mAb and opens a new approach to specifically measure APF in urine samples.

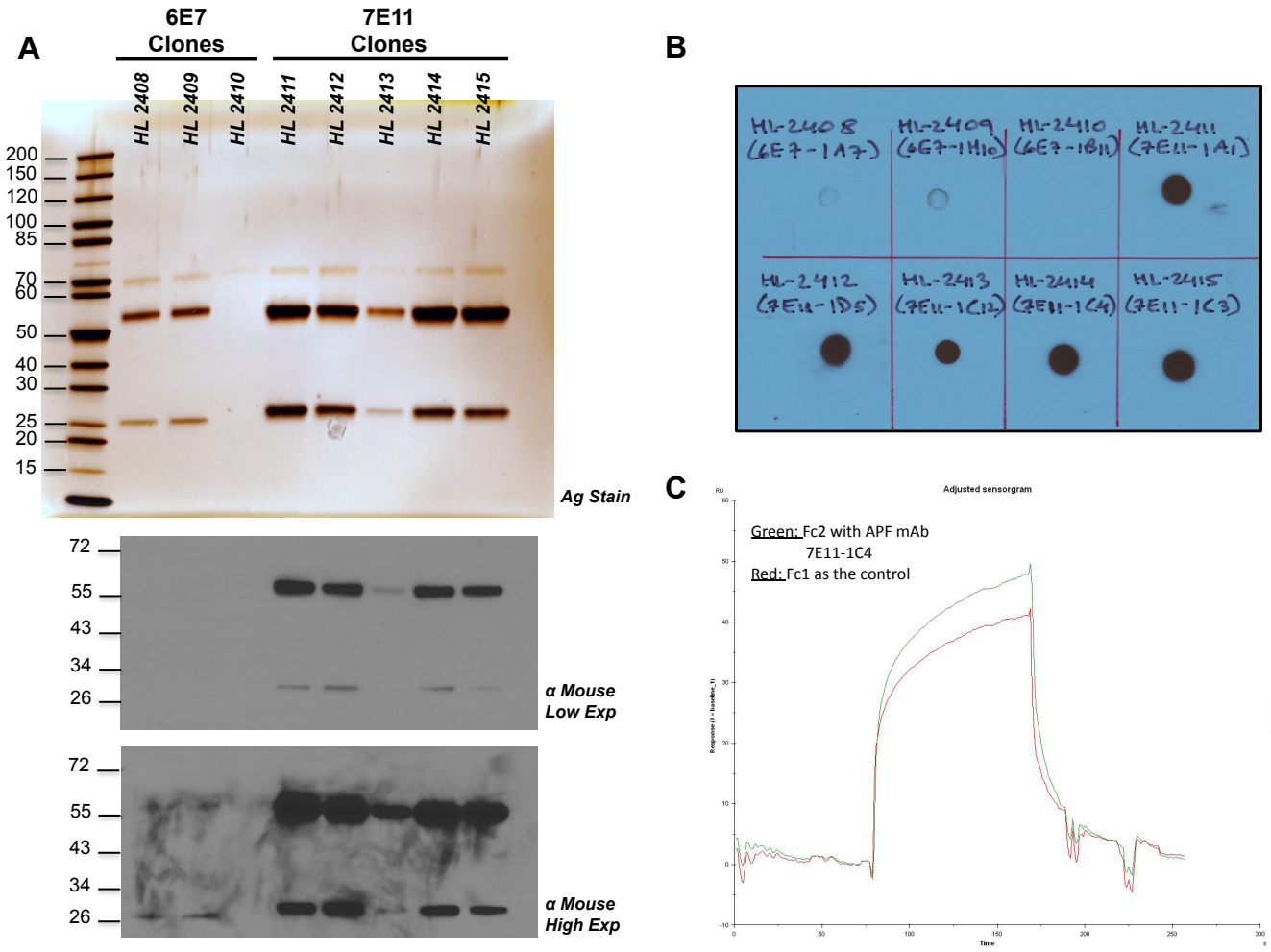


Figure 8. Confirmation of the affinity of cloned APF monoclonal antibodies. APF mAb hybridomas were subcloned by limiting dilution to isolate single clones with unique antigenicity against APF. At each stage of culture—96-, 24-, and 6-well—the cell culture supernatants were screened by ELISA and SPR to identify clones with high affinity and optimal kinetics for further cloning. During the final 6-well cloning stage, 12 clones each from the 7E11 hybridoma (IgG1, κ chain) and 6E7 hybridoma (IgG3, κ chain) were screened by ELISA and SPR. An Octet assay was also performed to determine their antibody quantitation and secretion. All 24 clones showed positive reactivity against APF, with varying binding kinetics. **(A)** Three 6E7 clones and five 7E11 clones were selected for large-scale production and purification. mAbs purified by protein A/G-beads are presented in the upper panel; the bottom panel shows western blot analysis results to confirm the antibodies. **(B)** Purified antibodies were screened for activity by dot blot. The membrane was spotted with 0.01ug KLH-APF and screened by 1:250 dilution of 6E7 APF purified antibodies and 1:500 dilution of 7E11 APF purified antibodies . **(C)** Further confirmation of the binding activity of the mAb to APF by SPR assay. 7E11-1C4 mAb was immobilized onto Fc2 channel of CM5 by amine-coupling as the ligand, the Fc1 was blank-immobilized as the control. 10uM of APF was used as the analyte for the binding, and the sensorgram is shown.

1b: *Optimization of conditions for sensor chip surface regeneration and binding reproducibility (months 1-12).* This will be accomplished concomitantly as we determine the optimal method for CKAP4 or APF antibody biosensor immobilization.

1c: *Characterization of the SPR-based assay using synthetic APF (months 12-18).* We expect that this task will be accomplished in the next year of the grant.

SOW – Specific Aim 2/Task 2: *Determine the ability of the SPR-based assay to detect APF in urine from patients with IC (months 1-36).* During this annual reporting period, effort was expended primarily

on Task 2a and 2b under Specific Aim 2. We have made substantial progress in the first year as detailed below.

2a: *Regulatory review and approval by Institutional Review Boards and DoD Human Research Protection Office (months 1-3):* This has been accomplished and approval has been received by all regulatory agencies.

2b: *Recruitment of human subjects for urine sample acquisition (months 4-24):* The recruitment of human subjects for urine sample acquisition has begun and is underway at the University of Pennsylvania. To date, 30 of the targeted 30 IC patients have been recruited and enrolled in the study at that site. Similarly, recruitment of human subjects at the Commonwealth Medical College has commenced and to date, 6 of the 30 age-matched, asymptomatic controls have been recruited and enrolled. There have been no issues or problems with patient recruitment at either site; however, our collaborator, Dr. Susan Keay expressed concern regarding the urine specimen collection cups being used in the study. We have temporarily ceased collecting urine specimens from recruited patients at both Penn and TCMC until her lab verifies that the composition of the collection cups (i.e., type of plastic) does not interfere with APF activity. We expect to have this resolved in the next few weeks.

2c: *Testing of biological urine specimens by the cellular proliferation assay (months 4-30).* We expect that this task will be accomplished in the next two years of the grant.

2d: *Testing of biological urine specimens by the SPR-based assay and comparison of results with cellular proliferation assay results (months 24-36).* We expect that this task will be accomplished in the next two years of the grant.

2e: *Statistical analysis (months 24-36).* We expect that this task will be accomplished in the final year of the grant once testing is completed.

KEY RESEARCH ACCOMPLISHMENTS:

- Received regulatory review and approval from IRBs at each study site and from the USAMRMC ORP and the HRPO.
- Established the infrastructure for the study by recruiting staff for the post-doctoral and research technician positions.
- Obtained required shipping infectious substances (IATA and DOT) certification for appropriate personnel.
- Procured shipping supplies for clinical specimens that are compatible with IATA regulations as well as other protocol-related supplies to be used at each study site.
- Developed standard operating procedure for collecting and processing fresh urine specimens across study sites.
- Commenced recruitment of patients for the study at both the University of Pennsylvania and at TCMC.
- Optimized rCKAP4 activity and immobilization on sensor chip surface with sufficient sensitivity to discriminate and quantitate APF by SPR in a purified system.
- Established an alternate methodology to increase sensitivity of the SPR assay using APF monoclonal antibodies as a biosensor.
- Developed and organized an Interstitial Cystitis Symposium at the The Commonwealth Medical College.

CONCLUSION:

In the first year of this grant award, we have successfully completed all of the pre-clinical study components for Aim 2 and received all of the necessary regulatory approvals. Developing the required infrastructure for the overall project, including the recruitment and hiring of a post-doctoral researcher and laboratory technician as well as establishing SOPs and procuring supplies to ensure consistency across sites, has been somewhat prolonged but ultimately successful. We have assembled an

excellent research team and are making considerable progress toward the development, characterization, and eventual testing of clinical samples by the SPR assay.

Our focus in this first year of the award has been on development of the SPR assay using CKAP4 as a biosensor to detect APF. To date, our results demonstrate that we have successfully optimized rCKAP4 activity and immobilization, achieving improved binding efficiency to APF using the CKAP4 ED 361-524 mutant. This binding efficiency is sufficient to detect and quantitate APF in a purified system. Our immediate goal moving forward is to test the specificity of this interaction in a non-purified system (i.e., urine) in order to characterize the assay further and test its diagnostic ability.

As an alternate, parallel approach to enhance the sensitivity of the SPR assay we sought to utilize a monoclonal anti-APF antibody as the biosensor in the SPR assay to detect APF as the analyte. We screened 13 primary hybridoma clones (previously generated by our laboratory) for APF sensitivity using the SPR assay and identified 6 clones with high binding affinity to APF. Following subcloning to isolate single clones with unique antigenicity and several rounds of screening, we purified five APF (7E11) monoclonal antibodies that displayed specific activity against APF-KLH in a dot blot assay. Importantly, the 7E11-1C3 clone showed specific binding to the active form of APF by the SPR assay. This significant result indicates the potential success of using an APF mAb as a biosensor and offers a new approach to specifically measure APF in urine samples. Testing of the other mAb clones is currently underway, and the detailed binding conditions are being optimized. Pursuing both biosensor strategies will enhance our success of developing an SPR-based assay that can detect and measure APF in urine from patients with IC with the goal of developing a non-invasive, point-of-care diagnostic test for IC.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

a. Abstracts:
   - Burzin Chavda, Jun Ling, and Sonia Lobo Planey. *Characterization of the binding of antiproliferative factor to cytoskeleton associated protein 4 by surface plasmon resonance.* American Society for Molecular Biology and Biochemistry Annual Meeting, San Diego, California, Sunday, April 25-29, 2014.

b. Presentations:
   - Interstitial Cystitis Symposium, The Commonwealth Medical College, 22-Feb-2014

INVENTIONS, PATENTS, AND LICENSES:

Nothing to report.

REPORTABLE OUTCOMES:

To date, there are no reportable outcomes, as the testing of biological urine samples for APF by the SPR assay has not yet commenced. However, an alternative approach taken to increase the sensitivity of the SPR assay has resulted in the production of five purified monoclonal antibodies that display specific activity against APF-KLH in a dot blot assay and specific binding to the active form of APF by the SPR assay. These antibodies are being further characterized and may offer a new approach and/or research tool to specifically measure APF in urine samples.

OTHER ACHEIVEMENTS:

Nothing to report.

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APPENDICES: Quad Chart

Validation of APF as a Urinary Biomarker for Interstitial Cystitis

PR121048 - Investigator-Initiated Research Award
W81XWH-13-1-0454

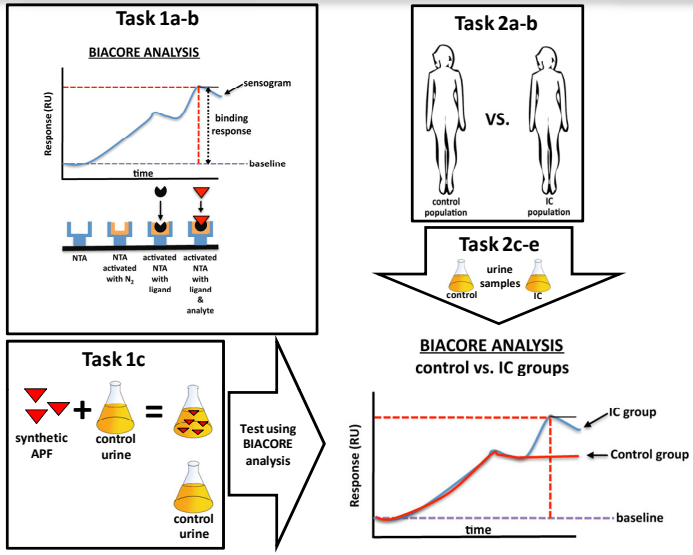
PI: Sonia Lobo Planey, Ph.D. **Org:** The Commonwealth Medical College **Award Amount:** $945,142.00

Study Aim(s)

Aim 1: *To develop and characterize a SPR-based assay employing a CKAP4 immobilized biosensor to detect APF.*
Aim 2: *Determine the ability of the SPR-based assay to detect APF in urine from patients with IC.*

Approach

To accomplish Aim 1 we will optimize rCKAP4 activity and sensor chip immobilization to achieve maximal CKAP4/APF binding efficiency and reproducibility. We will then determine linearity and range of the assay using known concentrations of synthetic APF spiked in urine from healthy donors. In Aim 2 we will determine the ability of the SPR-based assay to detect and quantitate APF in urine samples from patients with IC (45) versus non-IC, age-matched controls (45). Sensitivity and specificity of the SPR assay to detect APF will be determined by comparison with cellular proliferation assay results obtained by Dr. Keay. Statistical analysis will be performed to assess APF's utility as a diagnostic and/or prognostic biomarker for IC.



Testing of generated CKAP4-ED deletion mutants and APF monoclonal antibodies by SPR.

Timeline and Cost

Activities CY	13	14	15	16
Task 1a-b. Improved CKAP4/APF binding efficiency and reproducibility				
Task 1c. Characterization of SPR-based assay using synthetic APF				
Task 2a-b. Subject recruitment and acquisition of 90 urine specimens				
Task 2c-e. Sample testing by SPR assay and validation of APF				
Estimated Budget ($K)	**$50**	**$323**	**$313**	**$259**

Updated: (October 28, 2014)

Goals/Milestones
CY13 Goal – SPR assay development
☑ Optimization of rCKAP4 activity and immobilization
CY14 Goals – SPR assay characterization
☑ Improved CKAP4/APF binding efficiency and reproducibility
☐ Characterization of 20 samples using synthetic APF
CY15 Goals – Acquisition and testing of 90 biological specimens
☐ Testing for APF activity by cellular proliferation assay
☐ Testing for APF by SPR-assay
CY16 Goals – Statistical analysis and comparison of assay results
☐ First direct measurement of APF in human urine
☐ Assess APF's utility as a diagnostic biomarker for IC
Comments/Challenges/Issues/Concerns
- No concerns at this time with the scientific goals/accomplishments

Budget Expenditure to Date
Projected Expenditure: $323,000
Actual Expenditure: $273,440